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ABSTRACTS OF PAPERS

Sixteenth Annual Albert L. Tester Memorial Symposium, 10–12 April 1991[1]

The Albert L. Tester Memorial Symposium is held in honor of Professor Albert L. Tester, who, at the time of his death in 1974, was senior professor of zoology at the University of Hawaii. The faculty and students of the Department of Zoology proposed an annual symposium of student research papers as a means of honoring, in a continuing and active way, Dr. Tester's lively encouragement of student research in a broad range of fields in marine biology. Papers reporting original research on any aspect of biology are solicited from students at the university, and these papers are presented at the symposium, which takes place during the spring semester. Income from contributions to the Albert L. Tester Memorial Fund of the University of Hawaii Foundation is used to provide two prizes for the best papers by graduate students. Papers are judged on quality, originality, and importance of research reported, as well as on the quality of the public presentation. Judges include several members of the faculty of the Department of Zoology as well as winners of the symposium from the preceding year, when possible. In addition, a distinguished scholar from another university is invited to participate in the symposium as a judge and to present the major symposium address. This year John Maynard Smith of the University of Sussex participated in the symposium.

Evidence for Expression of a Sugarcane PEP Carboxylase Gene That Lacks a Canonical "TATA" Transcription Control Element[2]

HENRIK HORST ALBERT[3]

Most eucaryotic genes that have been studied contain as an essential part of their transcriptional control machinery a conserved consensus sequence, the "TATA" box. Few genes that lack this element have been shown to be transcribed. We have cloned a phosphoenolpyruvate (PEP) carboxylase gene from sugarcane that lacks a TATA element. In maize, PEP carboxylase has been shown to be encoded by a family of approximately five genes. One of these genes, which contains a TATA box, encodes the PEP carboxylase that is specifically involved in C-4 photosynthesis. This gene is expressed at high levels in a cell-type-specific manner in greening leaf mesophyll cells. The protein encoding region of our sugarcane gene is similar to that of the maize C-4 gene; however, in contrast to the highly expressed maize gene, transcription from the sugarcane gene is not detectable by northern blots or RNAse protection assays. Using primers based on the sequence of our cloned sugarcane gene in polymerase chain reaction (PCR) amplification and primer extension experiments, we now have evidence for transcription of this gene in roots, etiolated stems, shoots, callus, and leaves, and in greening and mature leaves. Transcript pools from this gene are larger in all of the nonphotosynthetic tissues than in greening or mature leaves. We conclude that this gene

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[2] The author is supported by a generous fellowship from the Hawaiian Sugar Planters Association.

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does not encode a C-4 sFific PEP carboxylase, and that the absence of a TATA box may be consistent with low-level constituitive transcription.

D-Glucose Transport in Lobster Antennal Gland Epithelium[4]

RACHEL D. BEHNKE[5]

Brush border membrane vesicles were prepared from antennal gland labyrinth of American lobsters (*Homarus americanus*) and incubated in media of defined ionic and organic composition. Movement of radiolabeled glucose into the vesicles was observed, and thus transport of this substrate by the carriers embedded in the apical membrane of the antennal gland epithelium was monitored. D-glucose transport by the antennal gland labyrinth was found to be Na^+-dependent, with 2 Na^+ bound and possibly transported for every molecule of glucose transported by the carrier. An inwardly directed transmembrane Na^+ gradient could drive the transport, but similar gradients of other monovalent cations, such as K^+ and Li^+, were unable to stimulate transport above passive diffusion. Na^+-coupled transport was dependent on presence of a permeable anion, such as Cl^-, without which there was no transport above the level of passive diffusion. An inwardly directed transmembrane Cl^- gradient alone could not drive uphill transport of glucose, however. Phlorizin, a very specific inhibitor of Na^+-dependent D-glucose transport, significantly reduced transport even at very low concentrations (< 0.01 mM) relative to the amount of substrate present. Competitive inhibition of transport by monosaccharides similar to glucose was tested. D-galactose was the only sugar tested that had even a slight effect on transport. Therefore, Na^+-glucose transport in these membranes was concluded to be highly substrate-specific. The pH of the medium both inside and outside the vesicles was found to have a significant effect on glucose transport: a low pH inhibits transport, while a higher pH stimulates transport. Preliminary measurements of kinetic parameters were made by fitting the Michaelis-Menton equation to the data. The Kt of the system was estimated to be 0.314 mM D-glucose and the Jmax to be 425 pmole D-glucose/mg membrane protein/sec/mM D-glucose$_0$.

Disparate Regulation of Growth Hormone and the Two Prolactins of the Tilapia, *Oreochromis mossambicus*, in Response to Salinity[6]

RUSSELL J. BORSKI[7]

Prolactin (PRL) and growth hormone (GH) are thought to play significant roles in the freshwater (FW) and seawater (SW) adaptation of euryhaline, teleost fishes. These studies were undertaken to investigate whether long-term rearing of tilapia in FW and SW could differentially alter pituitary content and subsequent in vitro release of the two PRLs (188 and 177 amino acid residues) and GH in the tilapia. Fish were raised from the period of yolk sac absorption for 6 months in FW or SW. Both forms of PRL were present in significantly greater quantities in pituitaries of FW-reared tilapia than in SW fish. The reverse was true for GH. The relative content or ratio of the larger to smaller PRL (188/177)

[4] Supported by NSF DCB-8903615 (Gregory Ahearn) and Sigma Xi grant (to author).

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[6] Supported by NSF DCB 86-15659, UH Sea Grant College Program NA 85 M-DSG082:R/AQ-37, and State of Hawaii ADP 27152 to E. Gordon Grau.

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was greater (~1.5:1) in the pituitaries of FW-reared tilapia than that seen in the pituitaries of SW fish (~0.75:1). This pattern was maintained even after pituitaries had been incubated for 18–20 hr in vitro. Growth hormone release in vitro, GH cell activity, and the relative area of GH cells to the whole pituitary were greater in SW-reared fish compared to FW fish. The reverse was true for PRL. When FW-reared fish were transferred to SW and held for 2 months, the ratio of 188/177 PRL was reduced to that seen in fish that were reared in SW. By contrast, the 188/177 ratio of fish transferred from SW to FW for 2 months was increased to that seen in FW-reared fish. Overall. these studies indicate that the processing of the two PRLs and GH is differentially sensitive to environmental salinity. This disparate regulation of the two tilapia PRLs suggests that they possess distinct, but possibly overlapping actions.

Molecular Population Genetics for Conservation of Native Hawaiian Forest Birds[8]

ROBERT A. FELDMAN[9]

One of the central problems in conservation genetics is the analysis of genetic variablity in natural populations. Application of PCR technology and sequencing of mtDNA genes is making large-scale high-resolution studies possible. An important aspect of this technology is in the analysis of population genetic phenomena and its relevance to macroevolutionary events. This study has investigated the mtDNA sequence variability in the Hawaiian honeycreepers (Drepanidinae). The group is an example of a recent adaptive radiation, having evolved into more than 41 species and subspecies of which 15 are now extinct from anthropogenic causes. Sequence comparisons were made of the cytb region from 20 individuals of each of four species: ʻĀkepa, ʻAmakihi, ʻApapane, and ʻIʻiwi living on the island of Hawaii. Sequences were also determined for the rare endangered ʻAkiapōlāʻau and the Hawaiʻi creeper. Phylogenetic trees drawn from the sequence data indicate a grouping of ʻAmakihi, ʻApapane, and Hawaiʻi creeper to the exclusion of the ʻIʻiwi and the ʻAkiapōlāʻau, with the ʻĀkepa being the most distant species. The cytb region is useful in making interspecies comparisons of the Drepanidinae. It may be an appropriate region in comparing birds from other islands in addressing questions of island migration. My studies are now emphasizing the more rapidly evolving D-loop region for intraspecific comparisons.

Successive Clutches in the Black Noddy, *Anous minutus*[10]

VANESSA H. GAUGER[11]

The vast majority of Hawaiian seabirds have a clutch size of one, and all have been reported to raise at most a single brood per year. Variation in clutch size is a well-studied avian reproductive strategy; an alternative strategy may be to increase the number of broods raised per year. I studied Black Noddies nesting on Tern Island, French Frigate Shoals, in the Northwestern Hawaiian Islands, to determine if pairs attempt successive clutches within an annual nesting season. I also studied mate and nest fidelity, and the interactions between the first-brood fledgling and its parents as they attempted a successive

[8] Blood samples provided by Jaan Lepson and Leonard Freed are appreciated. Supported by a MacArthur grant to Leonard Freed, Rebecca Cann, and Sheila Conant.

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[10] I would like to thank Sheila Conant and the U.S. Fish and Wildlife Service for their support.

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clutch. Black Noddies exhibit high mate and nest fidelity, and nest almost year-round on Tern Island. To monitor reproductive success of identifiable pairs from 1987 to 1989, I applied unique color combinations of leg bands to nesting adults and their chicks. During the nesting season from November 1987 to October 1988, 54% of 56 monitored pairs fledged one chick and 36% fledged two successive chicks (about 5 months apart). During the 1988–1989 year-long nesting season, 52% of 75 nesting pairs fledged one chick, 37% fledged two successive chicks, and 4% fledged three successive chicks. These results indicate that successive clutches may regularly contribute to reproductive success in this population.

Corals Benefit from Territorial Defense by the Herbivorous Damselfish *Stegastes nigricans* in Moorea, French Polynesia

DEBORAH J. GOCHFELD[12]

Observations on the damselfish *Stegastes nigricans* in Moorea indicated that the territorial defense activity of these herbivorous fishes is beneficial to hermatypic corals growing within the territory. All fishes, including coral-feeding butterflyfishes, approaching *Stegastes* territories were vigorously chased from the territory. *Pocillopora damicornis*, a preferred prey item of butterflyfishes elsewhere, grew only outside damselfish territories on fringing reefs in Moorea. Coral diversity was higher inside damselfish territories than in adjacent areas outside territories. *P. damicornis* colonies transplanted outside damselfish territories were fed upon voraciously by a variety of butterflyfish species. Feeding rate of *Chaetodon trifasciatus* on transplanted colonies was initially very high ($x = 144 \pm 52$ bites/5 min). Feeding rate decreased significantly over time as accessible tissue was removed. Feeding rate on *P. damicornis* transplants was significantly greater than feeding rate on *Montipora* sp., the most abundant food source for coral feeders in this habitat. This study suggests that *P. damicornis* and other preferred coral species survive in the refuge of damselfish territories, whereas corals settling and growing outside territories experience heavy predation and their growth and survival are suppressed. This is further evidence that predation by coral-feeding fishes controls the distribution of certain preferred coral species, restricting them to areas in which they are protected from predation.

Behavioral Changes Associated with Protandrous Sex Reversal in the Anemonefish *Amphiprion melanopus*[13]

JOHN R. GODWIN[14]

Functional sex change involves profound alterations in controlling mechanisms from the cellular level in the gonads to behavior. The behavioral correlates of protandrous sex change were examined in *Amphiprion melanopus* to characterize the nature and time course of changes in behavior and determine how these might be associated with levels of circulating gonadal steroid hormones. Sex change was stimulated in the field by removal of the dominant female member of mated,

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[13] Supported by an NIMH predoctoral fellowship.

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site-attached pairs. Behavioral data were taken from videotaped behavioral samples and during presentations of a conspecific in "model bottle" experiments. Frequency of aggressive acts increased significantly over baseline (i.e., male) levels by 20 days into sex change. Aggression directed at experimental intruders also increased in frequency and changed in pattern to that characteristic of females. Blood androgen levels did not appear correlated with these changes: no differences were found in testosterone levels, and 11-ketotestosterone was undetectable. Shifts in estradiol-17_β levels were correlated with gonadal changes and increased over a similar time course as frequencies of aggressive acts. These data indicate that estradiol-17_β may play a role in both gonadal and behavioral reorganization during sex change in *A. melanopus*.

Modulation of Catalase Activity during the Enforced Induction of and on Revival from Anhydrobiosis in Nematodes[15]

ALANE T. GRESHAM[16]

There are a number of anhydrobiotic systems that are able to control their ageing process exquisitely during periods of environmental stress. Nematodes are essentially aquatic organisms that have adapted to extreme environmental conditions, such as dehydration, by entering into a dormant state termed anhydrobiosis. Once dormant, they can persist for years (over 50 yr in some species) with little or no reduction in viability, suggesting a complete arrest of normal senescence. Free radicals, which are extremely reactive molecules because of their unstable electronic configuration, are inevitably generated when tissue is exposed to air (oxidation) and light (photolysis). A single radical is capable of initiating chain reactions that produce a 1000-fold amplification of damage. Therefore, the nematodes' ability to survive anhydrobiosis indicates that they are able to withstand free radical attack, suggesting that these nematodes must possess specific mechanisms to prevent oxidation and photolysis. Protective antioxidants have been found to inhibit free radical generation and accumulation in other organisms by converting radicals into harmless intermediates before the development of deleterious biological effects; they are hence pivotal in the control of normal ageing. We have begun to study the role of catalase during the induction of and revival from anhydrobiosis in the nematode *Aphelenchus avenae*. Nematode samples were induced into anhydrobiosis sequentially (97, 93, 85, 75, 40% relative humidity) for 24-hr periods, before holding at 0% RH for 3 days; then rehydrated by exposure to 100% RH for 12 hr. Upon dehydration, catalase activity increased dramatically after 2 days at 0% RH, but declined gradually throughout anhydrobiotic induction in nematodes stressed in culture before drying. Rehydration in both groups of animals resulted in a rapid increase in catalase activity during the first hour before returning to baseline levels. These results suggest that catalase may be important in arresting the ageing process.

[15] This research was funded in part by the U.H. Center on Ageing, U.H. Research Council Seed Money Grant, and a graduate assistantship from the RCMI Program in Cellular and Molecular Biology.

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Ecological Significance of the Ant *Pheidole megacephala* in Mealybug Wilt of Pineapple[17]

GARY C. JAHN[18]

Gray pineapple mealybugs (GPM), *Dysmicoccus neobrevipes* Beardsley, cause wilt disease of pineapple. When big-headed ants (BHA), *Pheidole megacephala* (F.), are eradicated from pineapple fields the GPM population declines and wilt disease is brought under control. To determine why BHA are vital to GPM survival, three hypotheses were tested: BHA (1) protect GPM from natural enemies, (2) provide sanitation for GPM by consuming honeydew, and (3) distribute GPM. Field and laboratory experiments suggest that BHA suppress predator populations, which allows GPM to proliferate. Thus, when ants are eliminated from a pineapple field with insecticides, the natural enemies remaining in the field actually suppress the GPM population. In the absence of BHA, in the field and laboratory, GPM were not observed dying as a result of excess honeydew, indicating that sanitation is not an important function of BHA in GPM survival. Experiments in the laboratory and field provided no evidence of ant-mediated GPM dispersal. Sticky trap collections in Maui pineapple fields revealed that first-instar GPM are definitely wind-borne.

Emergence of Central Pacific Ocean during the Late Holocene: Evidence from a Fossil Coral Reef on Kauai[19]

ANTHONY T. JONES[20]

A Holocene reef complex, ca. 500 m inland from the present shoreline, has been dated from 3620 to 4670 yr B.P. Reef framework was in growth position above present coral growth limits at a maximum elevation of ~1.8 m in the Hanalei River estuary, Kauai, Hawaii. A patch reef, composed entirely of *Porites compressa* Dana, is exposed in the river. Fossil corals including *Pocillopora meandrina* Dana and *Fungia* sp. are present in the adjacent Waioli Stream at similar elevations. The magnitude and timing of the Kauai reef approximates the emergence of French Polynesia and Cook Islands. Ecological implications of the Holocene emergence should be incorporated in models of island and atoll biogeography. As a consequence, Darwin's Point would have shifted closer to the equator at the time of maximum ocean expansion.

[17] Contributions to this work by Dr. J. Beardsley, Dr. N. Reimer, and the staff of Maui Pineapple Inc. are appreciated. Supported by GACC.

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[19] Contributions to this work by G. Smith are acknowledged. Supported by a grant from the State of Hawaii to R. Grigg and from NOAA National Undersea Research Program to A. Malahoff and the Harold T. Stearns Fellowship.

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Social Interactions and Foraging Ecology of the Ornate Butterflyfish, *Chaetodon ornatissimus*[21]

RANDALL KOSAKI[22]

The Indo-Pacific butterflyfish *Chaetodon ornatissimus* is a conspicuous inhabitant of coral reefs in Hawaii. Field observations at Puako, Hawaii, indicate that monogamous pairs of this obligate corallivore reside in intraspecifically exclusive territories. Territorial defenses were infrequent but occurred at the boundaries of home ranges marked by the convex polygon technique. *Chaetodon ornatissimus* fed on all species of the corals present in its territories, but showed preferences for certain species by feeding on them out of proportion to their availability in the environment. A recent storm damaged 7 to 12% of the coral in the territories of three pairs of *C. ornatissimus*. After the storm, sizes of these territories and rates of aggressive interaction with neighbors increased significantly. No changes in territory size or foraging area were noted in three pairs whose territories sustained minimal (<2%) or no coral damage. The results of this "natural experiment" suggest that *C. ornatissimus* densities are at least partially mediated by food abundance.

17Alpha-methyltestosterone and Growth of the Tilapia, *Oreochromis mossambicus*[23]

TODD T. KUWAYE[24]

The goal of this study was to identify the optimal pattern of 17alpha-Methyltestosterone (MT) application using a dose of 10 mg MT/kg of feed on growth of the tilapia, *Oreochromis mossambicus*, reared in fresh water and seawater. The experiment was designed with four treatments: control, continuous, early treatment, and delayed treatment. Each treatment was replicated in fresh water and seawater. Control fish were fed untreated feed whereas continuous MT-treatment fish received MT-treated feed throughout the experiment. Early MT-treatment fish were fed MT-treated feed for the first 2 months of the study and thereafter were fed untreated feed. Delayed MT-treatment fish received untreated feed for the first 2 months and thereafter were fed MT-treated feed. Seawater controls grew at twice the rate of the freshwater controls. Tilapia receiving an early or a delayed treatment of MT grew larger than the controls in both seawater and in fresh water. Nevertheless, the greatest growth was observed in fish that received a continuous treatment of MT. Results suggest that the optimal pattern of MT application for growth is continuous application of this hormone. Although the androgenic actions of MT may account for part of this increase in growth rate, our findings have shown that MT possesses greater growth-promoting activity than can be accounted for solely on the basis of its ability to masculinize tilapia.

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[21] Fieldwork at Puako was supported by the Hawaii Institute of Marine Biology.

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[23] Contributions to this work by D. S. Okimoto. S. K. Shimoda, R. D. Howerton, and E. G. Grau are appreciated. Supported by Sea Grant NA89AA-D-SG063 and State of Hawaii Aquaculture Development Program 29827.

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Plumage Variation in Female Hawai'i 'Ākepa (*Loxops coccineus coccineus*)[25]

JAAN KAIMANU LEPSON[26]

Plumage variation in the sexually dichromatic Hawai'i 'Ākepa has long puzzled naturalists and systematists. Extreme variation among males was assumed (and recently shown) to be the result of an unusually long delay in plumage maturation, but extensive differences among females were assumed to reflect fixed individual variation. Long-term study of banded females has revealed that this variation is also due to age, but is less consistent than among males. Sexual selection theory recognizes the expression of secondary sexual characters of males as correlated characters in females. Such correlation may be particularly strong in recently evolved populations, in which female coloration has not had sufficient time to stabilize around an optimum. The Hawai'i 'Ākepa shows a striking shift in male brightness and coloration relative to other subspecies and has evolved recently on the youngest island of the archipelago. Plumage variation also occurs among females in recently evolved populations of other dichromatic Hawaiian honeycreepers, suggesting a general pattern of correlation between plumage characters of males and females.

Analysis of Mitochondrial Control Region Sequences: Implications of Migration and Colonization within the Pacific[27]

J. KOJI LUM[28]

The two hypervariable regions of the human mitochondrial control region bounded by bases 15996 and 16400 and bases 29 and 408, and an additional noncoding region of the mitochondrial genome that shows a polymorphic length mutation, were enzymatically amplified from blood and hair samples from 30 individuals of Hawaiian, Australian Aboriginal, and Asian descent. Sequence differences among these individuals were used as character states to generate a dendrogram relating Pacific populations. Data from this analysis were compared to data obtained by others working with populations from Asia, Africa. and the Middle East. The result of these comparisons suggests a relatively recent migration of people into the Pacific in general and Polynesia specifically. This conclusion favors the "Noah's Ark" theory of human evolution over the "regional continuity" hypothesis. Sequence data from the second hypervariable region extracted from a 455 ± 115 yr old bone of Native Hawaiian origin are also discussed in reference to the effect of infectious disease on genetic diversity.

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[25] Funded by a NSF graduate fellowship and by a grant from the John D. and Catherine T. MacArthur Foundation to Leonard Freed, Rebecca Cann, and Sheila Conant.

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[27] Contributions to this work by O. Rickards and M. Pietrusewsky are appreciated. Supported by grants from NSF and HBI.

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Test of a Temporal-threshold Model of Polygynous Mating in Two Time Scales[29]

MARVIN LUTNESKY[30]

Temporal-threshold Model of Polygynous Mating (TMPM) predicts that polygynous females will compete for breeding order to avoid polygyny costs. It asserts that environmental cycles create optimal times to mate, but polygynous females can avoid polygyny costs by mating less synchronously (i.e., by dispersing their temporal pattern of mating). A polygynous angelfish. *Centropyge potteri*, was used to test the model. It is known to show both daily and monthly rhythmicity in its mating behavior. Females can spawn once per day on several days of the month. Peak mating is known to occur at dusk, in the week before full moon. Captive fish were observed in a tidal pond for a month. Six females were each given a male with whom to mate, and another six females were given a single male to share. Daily patterns of mating support the TMPM. Haremic females interfered with mating of other females significantly more often before mating. The variance in mean time of mating was significantly greater for haremic females than for paired females. The time of mating analyzed was adjusted for the male's "handling time," thus the statistic reflects time a female spent choosing to mate, not time waiting because the male was mating with another female. Monthly patterns of mating partially support the TMPM. Haremic females were significantly less synchronous in mating than paired females, but there was no significant difference of variance in mean day of the month for mating. Results of competition manifest themselves another way in this time scale: subordinate haremic females spawned significantly less often than paired females.

Advantage of Juvenile Coloration in *Labroides phthirophagus*

JEFFREY MAHON[31]

Juvenile reef fish commonly have a color pattern that is different from that of the adult. Hypotheses that address the delayed plumage maturation in young male passerine birds can account for this phenomenon in fishes. Juvenile coloration may minimize aggression from conspecifics: resident adults view an animal with adult coloration as a potential competitor for limited resources (e.g., food access, mates). Juveniles with special coloration would be less of a threat and thus receive less aggression from the resident. The Hawaiian cleaning wrasse, *Labroides phthirophagus* (a protogynous hermaphrodite in which all juveniles are females), was used in this test because the juveniles show a different color pattern and can quickly switch between adult and juvenile color patterns. Four juveniles (3–4 cm TL) switched to adult coloration when placed with an adult male, and to juvenile coloration when placed with an adult female. This suggests that juvenile coloration may reduce aggression in the presence of adult females, as the adult coloration may signify a competitor for mates. Males may treat the adult coloration as an indicator of an additional female for the harem. *Dascyllus albisella*, a common reef fish, was used to test if adult or juvenile coloration was preferred. Preliminary data suggest a preference for cleaners with adult coloration. This indicates that remaining in the juvenile color pattern may reduce the cleaners' access to food.

[29] My coauthor is Randall Kosaki, and this work was supported by Sigma Xi.

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Genetic Relationships within Species Complexes of Indo-West Pacific Butterflyfishes[32]

W. Owen McMillan[33]

The tropical Indo-West Pacific marine environment is spectacularly diverse. However, there is little consensus as to the evolutionary mechanisms generating diversity in that region. We explored speciation within the Indo-West Pacific by examining genetic relationships among members of two species complexes of butterflyfishes (genus *Chaetodon*) Both complexes contain a single Indian Ocean species and three Pacific species. We have sequenced a 650 base-pair segment of the mitochondrial cytochrome b gene from five individuals of all members of one complex and four of the five members of the other. The two complexes differed from each other by at least 12% (corrected for multiple changes at nucleotide positions) of their cytochrome b sequence. Within complexes, levels of genetic differentiation were much lower, ranging between 0 and 2.8% difference. There is a strong similarity in patterns of genetic relatedness among members of each species complex. In both complexes, the Indian Ocean species are clear outgroups to all Pacific species. Genetic relationships among the Pacific representatives are much less clearly defined. In both complexes, genetic differences between Pacific species are very small (between 0 and 1.2%) and are often exceeded by differences between individuals of the same species. This patterning of genetic relatedness is predicted if speciation occurred when once widespread ancestral populations were fractured synchronously as a result of widespread environmental changes such as recent change in sea level.

Role of the Sperm Surface Protein Bindin in Maintenance of Reproductive Isolation between Hawaiian Sea Urchins[34]

Edward C. Metz[35]

Strong barriers to cross-fertilization exist between closely related species of sea urchins in the genus *Echinometra* from Hawaii. Sperm-egg interaction was examined using light and electron microscopy to determine which of the steps of normal fertilization are not functioning properly in crosses between species. Equivalent gamete concentrations and times of gamete interaction were compared. The number of sperm capable of undergoing the acrosome reaction on heterologous eggs was found to range from 65% to 98% of the number undergoing the acrosome reaction on homologous eggs. The number of sperm attaching to heterologous egg surfaces was found to be only 23–30% of the number attaching to homologous egg surfaces. The barrier to cross-fertilization between Hawaiian *Echinometra* species primarily involves attachment and fusion of sperm

[32] Research was supported by The Lerner-Gray Fund for Marine Research and Sigma Xi to W. O. McMillan and NSF BRS 9000006 to Stephen R. Palumbi.

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[34] Electron microscopy by Hiroko Yanagimachi is much appreciated. Supported by NSF BSR 9000006 to Stephen R. Palumbi, and NSF predoctoral fellowship to E. Metz.

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and eggs. The sea urchin sperm surface protein bindin is involved in both of these events. A fragment of bindin coding DNA sequence was obtained from the two Hawaiian urchins. Comparison of sequences revealed a high ratio of coding to silent nucleotide substitutions, suggesting that selection for variability occurred. This phenomenon has been documented in other cell surface recognition proteins.

Patterns of Evolution in Love Songs of Koa-feeding Planthoppers[36]

CAITLIN E. O'CONNELL[37]

Patterns of population divergence are proposed for the monophagous complex of delphacids in the genus *Nesosydne* feeding on *Acacia koa*. There are four species in this complex. Several species have overlapping habitats with at least one other species. Two characters, male genitalia and mating song morphologies, were examined to determine phylogenetic relationships among the species in this group. An analysis of temporal patterns in the songs among populations revealed species-specific characteristics. The putative ancestral species display a complex song pattern from which the songs of three other species can be derived from the variable region of the ancestral song. It is suggested that sexual selection played an important role in the species-specific song patterns observed in this group and that the differentiation in the mating songs may not necessarily have evolved as a result of selection for isolation barriers between species.

Antifungal Activity of Aqueous Extracts of Hawaiian Lobelioids: Possible Effects on Egg Laying of Hawaiian *Drosophila*[38]

RICHARD PALMER[39]

Hawaiian flies of the genus *Drosophila* (Diptera) utilize rotting tissues of endemic Hawaiian lobelioids (Campanulaceae, tribe Lobelioideae), in which they lay their eggs. Larvae feed, presumably, on the microorganisms growing on the rotting tissue, and at the same time seem to gain protection from fungal contamination. Protection may come from the lobelioid itself or from the associated microorganisms. Initial investigations indicated that microbial growth promotes this antifungal activity. Of numerous extracts of lobelioid tissue, only those preparations not sterile-filtered showed in vitro fungal inhibition. This suggests that the microbial growth inhibits fungal contamination by synthesizing a fungal toxin de novo or metabolizing a constituent of lobelioid tissue/latex that in turn inhibits fungal growth.

[36] Supported by a Hawaii Bishop Research Institute grant.

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[38] Many thanks to Drs. Gerald Carr for sponsorship, George Wong for guidance in fungal biology, and Ken Kaneshiro for inspiration and support, and also to Linden Doescher, Bill Perreira, and Kelvin Kanegawa for their insights and unfailing cooperation.

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Arthropods, Habitats, and Conservation of Insectivorous Hawaiian Forest Birds[40]

ROBERT W. PECK[41]

Loss and degradation of habitat has had a devastating effect on the distribution and abundance of native Hawaiian forest birds. Census studies have positively correlated vegetative structure and composition with bird abundance, leading to the assumption that greater canopy cover and native understory represent superior habitat. However, some of the highest bird densities of insectivorous species occur in parkland forests associated with cattle ranching. The relation between forest structure and arthropod diversity and abundance was investigated in contiguous tracts of different structure at Hakalau Forest National Wildlife Refuge on Mauna Kea, island of Hawaii. Arthropods were sampled using light and malaise traps, terminal branch clipping, and sweep netting. Preliminary results suggest that the less-disturbed tract has greater diversity and abundance of arthropods, but the larger trees in the parkland forest may provide more prey in the foraging microhabitats used by insectivorous birds. This finding has implications for the conservation and management of habitat-dependent insectivorous birds.

Activity Patterns and Space Utilization of Scalloped Hammerhead Pups, *Sphyrna lewini*, in Kaneohe Bay, Hawaii[42]

JOHN D. PETERSON, JR.[43]

Kaneohe Bay is a pupping ground for *Sphyrna lewini* where the juvenile sharks probably represent a significant fraction of the biomass of large carnivores. Their activity may have important effects on the energy budget of this ecosystem. Ultrasonic telemetry was used to determine movements of six pups during the period from July to October, 1990, with intermittent multi-day tracks (1–12 days). The longest segment of continuous contact lasted 77 hr. Transmitters were implanted using forced ingestion in the field. The overall daytime mean rate of movement was 0.60 km/hr; the nighttime rate of 0.67 km/hr was significantly higher. Activity rate during the crepuscular hours was higher (although not significantly) than at other times. The maximum measured rate of movement was 1.43 km/hr. Observed total activity space of an individual ranged from 0.46 km^2 to 3.52 km^2 ($x = 1.12$ km^2) as determined by the maximum convex polygon method. Overall nocturnal activity spaces were larger than diurnal activity spaces. A grid-square analysis of intensity of usage indicated a disproportionately high occupancy of a "core area" during the day. Overlap on consecutive days indicated a high degree of site attachment. Total overlap between all sharks was higher for the diurnal than for the nocturnal activity

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spaces. Total activity spaces were limited to waters below the 9-m depth contour. Pups show a repeatable, diel pattern of "refuging": aggregating in a core area of their home range during a less active diurnal phase and dispersing outward over a larger arena at night when they are more active and presumably foraging. Energetic and antipredatory benefits may result from this behavior.

Reproductive Biology of *Asterropteryx semipunctatus* (Pisces: Gobiidae) in Kaneohe Bay, Oahu

LISA A. PRIVITERA[44]

This study was conducted to determine the seasonality of spawning and basic reproductive parameters of the goby *Asterropteryx semipunctatus* in Kaneohe Bay, Oahu. Fish were collected at monthly intervals for 1 yr to determine size at maturity, size composition of spawners, fecundity versus size relationship, and spawning frequencies. Field data were compared with spawning in laboratory aquaria. Both sexes begin to mature at 19 mm standard length (SL); almost all fish over 24 mm SL were mature and in spawning condition. Mature fish were found in all months of the year. Batch fecundities determined from ovarian analyses ranged from 138 to 2210 eggs and were comparable to clutch sizes of fish spawned in the laboratory. No strong relationship exists between size and fecundity. Condition factor, however, appears to be a better predictor of fecundity. Males are significantly larger than females and have longer third dorsal spines relative to body length. All mature females possess one to seven yellow spots on the caudal peduncle. Occurrence of yellow spots in some small males and incidents of sneaking behavior in the laboratory suggest a possible female mimicry mating tactic.

Clearance of Thyroid Hormones from the Tilapia, *Oreochromis mossambicus*, and Effects of Triiodothyronine (T_3) on Intestinal Nutrient Absorption in Freshwater- and Seawater-adapted Tilapia[45]

BENNY RON[46]

Effects of graded doses of T_3 on proline and glucose absorption by the anterior intestine of tilapia, *Oreochromis mossambicus*, adapted to fresh water (FW) and to seawater (SW) were examined in this preliminary study. Nutrient absorption was measured using the everted sleeve technique. Reductions in both length-specific glucose uptake (uptake per cm of intestine; LS) and weight-specific glucose uptake (uptake per mg of intestine; WS) were observed in the SW tilapia compared to their FW counterparts. Passive proline uptake, both LS and WS, were not altered by T_3 or salinity treatments. Although there were no significant differences among T_3 and between salinity treatments in either LS or WS active proline uptake, a trend toward lower active

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proline uptake in the highest T_3 treatments was seen. In addition, clearance rates for thyroid hormones were determined by injecting [^{125}I]thyroxine (T_4) or [^{125}I]T_3 into the heart and monitoring the disappearance of the tracer from the circulation by sampling blood from the caudal vasculature. Similar techniques were employed to determine plasma volume, using injections of [^{125}I] human serum albumin. The plasma volume of male tilapia has been found to increase linearly with increase in body weight. Preliminary results demonstrate that both T_4 and T_3 are cleared rapidly from the male tilapia.

Occurrence and Diets of Juvenile *Caranx ignobilis* and *Caranx melampygus* (Teleostei: Carangidae) in the Hanalei River Estuary, North Kauai, Hawaii

GORDON C. SMITH[47]

Estuaries are known to provide juvenile nursery habitat for many species of fish that inhabit neritic marine environments as adults. In Hawaii, juvenile *Caranx ignobilis* and *C. melampygus* occupy estuarine habitats opportunistically before moving to nearshore ocean reefs as adults. This study describes the extent of estuarine habitat available in the Hanalei River, the relative abundance of jacks present in the estuary, and the diets of those fish as determined by analysis of stomach contents. Salinity measurements taken over a period of 19 months indicated that the upstream extent of brackish water ranged from the mouth to nearly 5 km upriver. Ingress of seawater into the estuary was more closely related to stream flow volume than to tidal variation in sea level. Beach seine collections taken over 17 months produced overall catch rates of 0.63 fish per haul for *C. ignobilis* and 0.64 fish per haul for *C. melampygus*. Sizes ranged from 51 to 169 mm fork length (FL). Juvenile jacks were visually observed on snorkel transects over the full range of mixohaline conditions. Visual observations were of fish between 80 and 230 mm FL. Analysis of stomach contents indicated that the two species of jacks ate much the same spectrum of food items. Fish predominated as prey items in the diets of both species, but *C. melampygus* consumed benthic crustaceans more frequently, especially isopods and tanaids.

Trophic Link between Stream Discharge and Spawning Patterns in a Coral-reef Fish

WILLIAM A. TYLER III[48]

Hypotheses accounting for temporal reproductive patterns in marine organisms have traditionally focused on the biology of their pelagic offspring. Offspring survival may be enhanced by synchronized release with a number of environmental phenomena including seasonal cycles in upwelling, lunar cycles in nighttime illumination and currents, or diel cycles of tidal currents or photoperiod. Much less emphasis has been placed on variables that may influence adult reproductive strategies. Inter-annual variation was revealed from long-term spawning records of a plank-

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tivorous reef fish, *Abudefduf abdominalis* (Pomacentridae), from two patch reefs in Kaneohe Bay, Oahu, Hawaii. Population-level spawning patterns, ranging from lunar and 5-day cycles to acyclic spawning, were not consistent with hypotheses requiring synchronization with environmental cycles. However, long-term increases in monthly stream discharge and reproductive output were correlated at both sites, suggesting that nutrient input and food availability may affect spawning patterns. A field experiment provisioning adults with commercial fish food showed that increasing adult food abundance in nesting colonies can increase reproductive output and the frequency of spawning peaks when compared to control colonies. These results support the hypothesis that fluctuations in adult food abundance can affect population-level spawning patterns and that ecological variation affecting the adult must be considered when examining temporal reproductive patterns in marine organisms.

Endemic Hawaiian Species Radiation in the Neritidae[49]

CATHERINE R. C. UNABIA[50]

The high level of endemicity of the gastropod family Neritidae in Hawaii (8/11 species: 72.7%) is surprising because most have planktotrophic veliger larvae that should act as good dispersal agents, preventing differentiation of populations leading to speciation. Two species of *Neritina* (brackish to fresh water), two *Theodoxus*, three *Neritilia*, and the only species of *Smaragdia* (marine: seagrass associate) are all endemic. Only the three species of *Nerita* (marine intertidal) are not. In genera with more than one endemic species, did the species radiate from a single source, or did they immigrate and differentiate separately? Examination of radulae using SEM of these and closely related taxa suggests that Hawaiian species radiation occurred in two of the three neritid genera with sets of endemic Hawaiian species. *Neritina granosa* and *N. vespertinus* have similar winged shells, but radulae show that each is more closely related to other species outside Hawaii. *Theodoxus cariosus* has a winged shell like the *Neritina* species, but a very different radula. The radula of its Hawaiian congener, *T. neglectus*, is very similar, especially in the central portion, suggesting common derivation. Two of the three *Neritilia* species, recently discovered in small anchialine lava pools, have minor tooth shape differences. Although the shells are quite different, this combined with the presence of ridges on the shell and the lack of eyes in both suggests a common origin, probably *N. hawaiiensis*.

Regeneration of a Native Diverse Mesic Forest in Pahole Natural Area Reserve, Oahu, Hawaii: Implications for Conservation Biology[51]

PATRICIA C. WELTON[52]

Study of plant community organization can indicate patterns of species dynamics useful for predicting trends that can help in natural area management. The study area was designated in 1974 to protect lowland mesic and dry native communities. Since then there has been canopy dieback of two dominant tree species, *Metrosideros polymorpha* and *Syzigium sandwicense* (both Myrtaceae). Die-

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back observed on Hawaiian islands has been hypothesized as a mechanism of forest regeneration. Initial invasion by alien species threatens the survival of many native species. Community composition and the nature of species interaction has been studied along a gradient of canopy decline and disturbance by cattle. Native species are regenerating in areas where there was canopy decline but relatively less disturbance, while alien invaders were more evident in more disturbed sites.

Buoyancy of Deep-sea Sharks

BRADLEY M. WETHERBEE[53]

Deep-water sharks are believed to approach neutral buoyancy, presumably as a means of conserving energy in a nutrient-poor environment. In previous studies, the density of some deep-sea sharks has been found to vary between species and among different-sized individuals of the same species. Buoyancy of seven species of deep-water squalids and one scyliorhinid shark collected in New Zealand waters was determined, and factors that may be responsible for observed differences within and between species were evaluated. Density of sharks was calculated from measurements of their weight in air, weight in water, and volume. Liver density and the area of surfaces capable of providing hydrodynamic lift were also measured. All species of deep-water sharks were neutrally buoyant or nearly so; weights in seawater were -3.5 to $+2.0\%$ of their weights in air. There was considerable variation between species, but buoyancy was relatively constant for different-sized individuals within species. Size of fins and tail were not correlated with buoyancy, and these hydrodynamic features appear to be relatively unimportant. Liver size was a fairly constant fraction of body weight within species, typically about 20%. Liver density was inversely related to density of the liver-free body. Relationships of buoyancy with hydrodynamic lifting surfaces, body size, and liver size for the scyliorhinid shark were similar to those of shallow-water sharks, and differed from those of the deep-water squalid sharks. Despite minor variations in features that contribute to increased buoyancy, deep-water sharks of different species and sizes all maintained densities similar to that of the surrounding seawater.

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